PATENT
Docket No.: PIT-010CIP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Michael B. CHANCELLOR et al.		
Serial No.	:	09/549,937	Art Unit:	1635
Filed	:	April 14, 2000	Examiner:	Brian A. Whiteman
For	:	**Soft Tissue and Bone Augmentation and Bulking Using Muscle-Derived Progenitor Cells, Compositions and Treatments Thereof**		

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Tracy W. Cannon, M.D. under 37 C.F.R. §1.132

I, Tracy W. Cannon, M.D., hereby declare and state that:

1. I am a member of the faculty in the Department of Urology at the University of Pittsburgh School of Medicine, Pittsburgh, PA. As part of my responsibilities as a physician and faculty member, I perform clinical studies, and direct and carry out research, primarily in the field of urological dysfunction. Over the past two years, my research has particularly focused on developing and testing new treatments for stress urinary incontinence (SUI). During this time, I have been conducting research with Drs. Michael Chancellor and Johnny Huard in the areas of muscle derived cell (MDC)-based treatments for SUI using mouse and rat animal model systems. A copy of my Curriculum vitae is attached as Exhibit 1.

2. I have read and am familiar with the subject matter of the above-identified patent application (hereinafter, "the Chancellor application") and with the MDC work of Dr. Chancellor and Dr. Huard, including the method of isolating MDC and the method of using MDC in treatments such as bulking and augmenting tissue and treating tissue weakness and dysfunction. I have been told and understand that the claims of the Chancellor application have been rejected for lack of enablement.

3. In my opinion, the level of skill in the art of cell isolation, cell culturing and plating techniques, and cell-mediated therapy, i.e., the introduction of cells into animal hosts for *in vivo* treatment, study and analysis, was high at the time the Chancellor application was filed. For example, researchers in this scientific field, which includes myself, would have had a medical degree (M.D.) and/or a doctorate degree (Ph.D.) in an area such as cell biology, molecular biology, urology, or in a related field such as physiology and at least several years of research and/or clinical experience following completion of the advanced degree. In addition, those of ordinary skill in the art would also have had at least one or more years of actual experience in the field of cell mediated therapy and treatment.

4. Based on my knowledge in the field and my own personal experience, I declare that one having skill in this art is able to attain MDCs using muscle cells from a variety of mammalian sources, such as mice, rats, rabbits, pigs and humans, to name but a few. Obtaining a muscle cell suspension from an appropriate muscle tissue source does not require excessive experimentation. In addition, no undue or excessive experimentation is involved for a person skilled in the art to

carry out the plating technique to achieve an end population of isolated MDCs for use in the methods of tissue bulking, augmentation and treatment of tissue weakness or dysfunction in a host mammal, as described in the Chancellor application. Specifically, my direct experience and knowledge of the use of MDC and related techniques are evidenced in the publication, i.e., J.Y. Lee et al., 2003, "The Effects of Periurethral Muscle-Derived Stem Cell Injection on Leak Point Pressure in a Rat Model of Stress Urinary Incontinence" *Int. Urogynecol. J. Pelvic Floor Dysfunct.*, 14(1):31-37, (attached as Exhibit 2), on which I am a co-author and which describes the utilization of MDC according to the invention of the Chancellor application. As described in this publication, rat MDC injected into the denervated urethral sphincter muscle of anesthetized rats caused an increase in dorsolateral skeletal muscle masses with variable fiber orientation at the injection sites after four weeks. It is further shown that MDC isolated from rats survived in the lower urinary tract, developed into myofibrils to increase the presence of skeletal muscle fibers around the urethra, and increased the leak point pressure in denervated rats following periurethral injection, thereby bulking and augmenting the tissue and ameliorating the dysfunction. In addition, I am a co-author of another article that has been accepted for publication in the journal *Urology*, namely, T.W. Cannon et al., "Improved Sphincter Contractility after Allogeneic Muscle Derived Progenitor Cell Injection into the Denervated Rat Urethra". (attached as Exhibit 3). The work described in this article shows that MDC survive and serve as allogeneic transplants to restore defective urethral sphincter function in urethral tissue with minimal inflammation.

5. Based on my knowledge in the field and my own personal experience, I declare that the results obtained using MDC techniques for tissue bulking and augmentation in mice and rat recipient hosts, as described in the Chancellor application, would be reasonably predictive of similar types of results using MDC techniques in humans or other mammals. The results obtained using mouse MDC and mouse and rat models are suitable and accepted as being predictive of the results that would be expected to be seen in similarly-treated human subjects. The use of animal models as reasonable predictors of treatment outcome in humans is known and accepted in the art. A number of reports in the scientific literature at the time of the Chancellor application involve studies using non-human experimental animals, or non-human and human tissue or organ samples, and show that similar results are obtained for the study animals and humans. Such reports demonstrate that studies using mice, rats and rabbits, e.g., as clinically pertinent animal models, are reasonably predictive of effects and results obtained for humans. For example, rat, monkey and human bladder samples were examined for mRNAs and receptor binding sites for the alpha 1a-adrenoceptor (1A-AR) subtype to determine whether 1A-AR agents are useful in treating urinary smooth muscle related disorders (P.D. Walden et al., 1997, *J. Urology*, 157:1032-1038), (attached as Exhibit 4). Another publication describes the use of rabbits and rats as animal models of human bladder dysfunction to elucidate the development of such dysfunction and to reduce or prevent bladder dysfunction in humans based on protection of the bladder from ischaemic damage (R. Buttyan et al., 1997, *Eur. Urol.*, 32(suppl. 1):32-39), (attached as Exhibit 5). A study of the effects of ginkgo biloba extract on human and rabbit

corpus cavernosum tissue was performed to determine if subfractions of this extract having a relaxing effect on corpus cavernosum tissue could be used as a therapy for impotence, for example. (J-S. Paick and J.H. Lee, 1996, *J. Urol.*, 156:1876-1880), (Exhibit 6). In another report, mice having targeted deletion of the gene encoding neuronal nitric oxide synthase exhibited bladder disorders which modeled clinical disorders in humans. (A.L. Burnett et al., 1997, *Nature Medicine,* 3(5):571-574), (Exhibit 7). Species similarities have been reported in connection with nitrergic control of peripheral sympathetic responses in the corpus cavernosum, particularly in rabbit and human tissue. (S. Cellek and S. Moncada, 1997, *Proc. Natl. Acad. Sci. USA*, 94:8226-8231, (attached as Exhibit 8). Yet another publication reports that a comparison of streptozotocin-diabetic rats and a human diabetic was undertaken to determine that impairment in vasoactive intestinal polypeptide (VIP) immunoreactivity in nerves may play a role in impotence in diabetic males, as well as to determine that streptozotocin-treated rat may be a useful experimental model for diabetic autonomic neuropathy. (R. Crowe et al., 1983, *Diabetes*, 32:1075-1077), (attached as Exhibit 9).

6. Based on my knowledge and work in this field and my own personal experience, I declare that one skilled in this art would be able to reasonably extrapolate from the use of mouse and rat MDC in mouse and rat hosts that MDC from a human muscle source, for example, skeletal muscle, can be used at levels that would support tissue augmentation and bulking, and treatment of tissue weakness or dysfunction in human hosts. The results obtained by the MDC

work of Drs. Chancellor and Huard are most encouraging for the use of MDC techniques in humans in need of MDC treatment, such as patients suffering from SUI, as an example.

7. Based on my knowledge and work in this field, MDCs can be used to bulk and augment tissue and to treat tissue weakness and dysfunction with minimal concern about immune rejection, poor cellular survival and limited spread of MDC after introduction into a host, because the MDC isolated as described in the Chancellor application display survival for weeks and months following introduction into a host, proliferate, and are not typically rejected by the host, due to their probable immunoprivileged nature as early cells. See, for example, the publication of J.Y. Lee et al. (Exhibit 2).

8. Based on my knowledge of, and experience with, the MDC isolation technique and methods of using MDC, I have observed that MDC injected into a site, for example, a site of muscle tissue, in an animal can and do differentiate into cells of the appropriate tissue over time, e.g., within about 7-10 days, and survive in the area for longer periods of several weeks and months. See, for example, the publication of J.Y. Lee et al. (Exhibit 2).

9. Based on my knowledge and work in this field, and on my opinion and belief, I state that the Chancellor application provides a person skilled in this art with sufficient guidance to make and use not only mouse and rat MDCs, but also MDCs from other host mammals. The process of obtaining a starting suspension of muscle cells from a muscle cell source is known by those in the art; once these cells are obtained, no undue or excessive experimentation is involved in

carrying out the inventors' cell plating technique until an end population of non-fibroblast MDCs are present in the culture plates as described by the inventors and for use in the cell-mediated methods that are described and exemplified in the Chancellor application.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Chancellor application or any patent issued thereon.

Date: 7/21/03 By: _____
Tracy W. Cannon, M.D.